Assessment of in-situ natural and enhanced chlorinated ethenes degra-dation by use of isotopic and molecular biology techniques

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Main text (Abstract)	**Background** Chlorinated solvent contaminated sites continue to be a challenge in risk assessment and remediation. Source zone remediation has long been in focus in efforts to eliminate contaminant spreading and reduce risks. However, the often long and deep groundwater plumes continue to pose a risk, even where source remediation has significantly reduced the contaminant flux to the plumes. Hence, fate of contaminant plumes has recently gained focus. Natural attenuation may be sufficiently effective to contain the plumes with or without source remediation. In other cases this may be obtained through stimulation of the degradation processes through addition of donor and/or bio-augmentation possibly enhanced by the addition of sorbents or reactants. In-situ documentation of chlorinated ethenes degradation and quantitative determination of the effectiveness (including in-situ degradation rates) of natural and enhanced degradation is challenging. The potential for in-situ assessment is facilitated by the introduction of new techniques, including compound specific stable isotope analysis (CSIA) for documentation and multi element CSIA for process identification, and molecular biology techniques to document and quantify microbial species, their genes and activities responsible for biodegradation. The continued developments in their use further the potential. **Aim** The presentation aims, through examples, to illustrate and evaluate the benefits of applying these new techniques in risk and remedial effect assessments of chlorinated ethenes plumes. Important process understanding gained through research application of these techniques at real sites is also presented. **Examples** One of the most extensively studied chlorinated ethene plumes is a > 2 km long plume of PCE and chlorinated degradation products, that has migrated downgradient from the source zone PCE DNAPL contamination at the former central dry cleaning facility in Rødekro, Region of Southern Denmark. The source zone was subject to thermal (steam) source zone remediation in late 2006. The plume has not undergone active remediation. Natural degradation of cDCE and VC as well as PCE and TCE within the plume prior to source treatment was documented by line of evidence including stable isotopes in 2006-7 (Hunkeler et al., JCH, 2011). Enhanced degradation within the plume caused by the release of dissolved organic carbon during thermal remediation of the source zone (Figure 1) was documented in an integrated approach including dual element CSIA to distinguish biotic and abiotic processes as well as document degradation and molecular biological techniques to determine community composition and specific degrader presence and activity in 2014 (Badin et al., JCH, 2016). The analysis for microbial composition and specific degraders and their activity as well as dual stable isotopes has revealed high complexity in degradation processes and played an important role to substantiate the natural attenuation of the plume. More recent studies (2017-18) have focused on furthering the understanding of the degradation processes, including the relative importance of the genera *Dehalogenimonas* and *Dehalococcoides* and potential competing organisms (iron and sulphate reducers), as well as the evolution in natural attenuation and risk of the plume (Murray et al. 2018). A special emphasis has also been put on the potential for estimation of degradation rates in the plume in 2017 investigations.

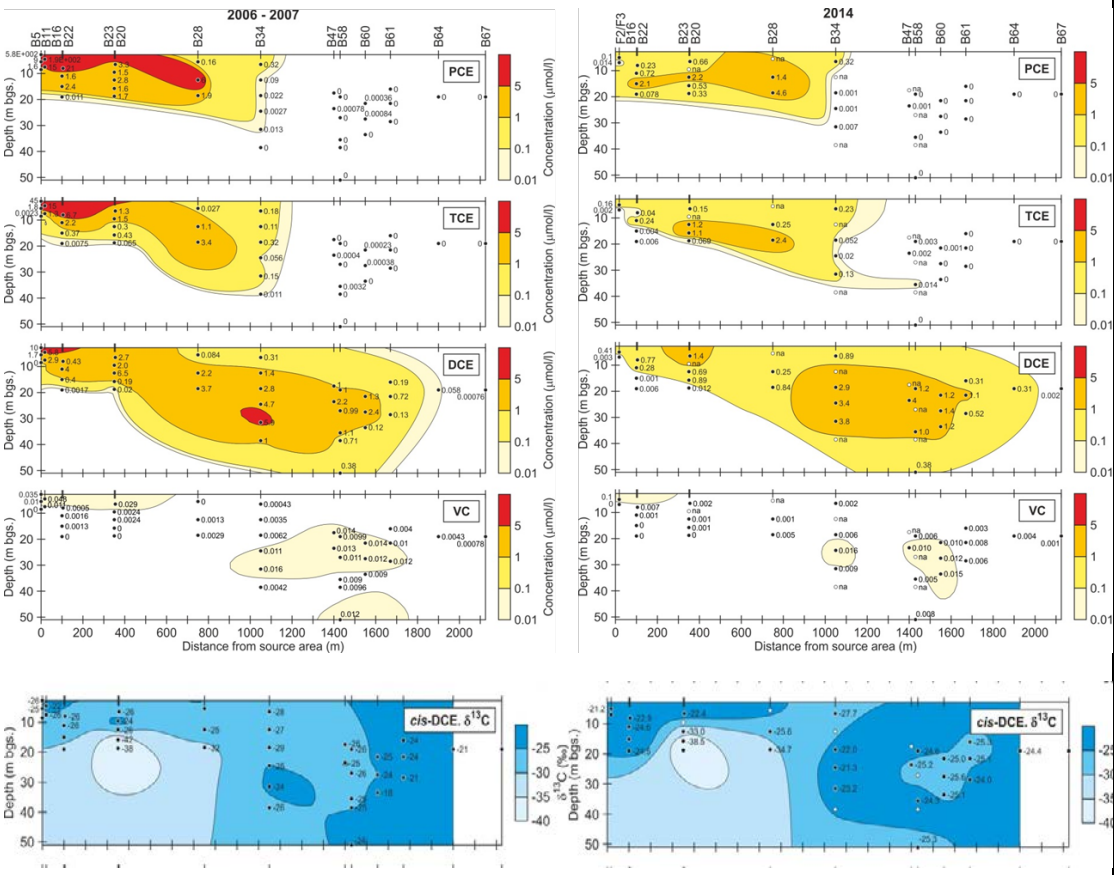

Figure 1. Chlorinated ethene concentrations and carbon CSIA data for cDCE for the Rødekro plume prior to and 8 years after the source remediation illustrating the concentration decrease and the increase in degradation documented by CSIA (Hunkeler et al. 2011; Badin et al. 2016).

This project is unique in the integrated characterization approach for line of evidence evaluation of the natural attenuation of cDCE and VC in the cDCE dominated plume and the monitoring of the effects of source remediation on plume natural attenuation.

The techniques are becoming more available to practitioners and are currently in use at a number of sites in the Capitol Region of Denmark where natural attenuation or enhanced biodegradation is evaluated. The conditions for and effectiveness of natural attenuation at these sites differ significantly from complete reductive dechlorination at relatively high rates near source zones (Ottosen 2017) to very limited degradation prior to remedial enhancement (Tuxen, 2018), illustrating the breadth of challenges facing use of the techniques.

The techniques are currently applied in a TCE plume treated with liquid activated carbon amendment combined with donor stimulation and bio-augmentation. The sorption of the TCE and its degradation products to the carbon challenges the application of traditional analysis as well as the new techniques to aqueous as well as sediment samples. The compound specific retardation and the bioactive treatment zone challenge the interpretation of the processes and remediation effectiveness. This site offers the first ever results where the techniques are applied under the complexity of strongly enhanced sorption and biodegradation (Ottosen et al. 2019?). We expect the new isotopic and molecular biology techniques and further development of these will be critical for the assessment of the remediation effectiveness.

Conclusion and perspectives

Techniques that determine microbial community composition, specific degraders presence and activity, and dual element CSIA have revealed high complexity in degradation processes and played an important role to substantiate the natural attenuation of plumes. Some of the techniques have become available to practitioners and are likely to significantly improve site diagnostics, risk assessment and effects of remedial measures. Use of the techniques is expected to be critical for the evaluation of process stimulation and differentiation in the ever more complex remediation schemes involving both physical, chemical and biological contaminant removal.

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